Management of Cotton Industry Waste Through Biotechnological Methods

A
RESEARCH PLAN
SUBMITTED

FOR THE PARTIAL FULFILLMENT OF

Doctor of Philosophy (Ph.D.)

Supervisor

Dr. K. P. Sharma

Submitted by

Ritu Saharan

Department of Science
Faculty of Arts, Science & Commerce
MODY UNIVERSITY OF SCIENCE & TECHNOLOGY
(Deemed University u/s 3 of UGC Act, 1956)
Lakshmangarh-332311, District-Sikar, Rajasthan (India)

Student. File Copy.

Introduction

Cotton industries are one of the largest polluter in the world as it utilizes various harsh and hazardous chemicals (alkali, acids, bleaching powder etc.). It generates a variety of toxic compounds in industrial waste water and results into high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values (Ghaly et al., 2014). Table 1 shows general characteristics of textile waste water and the standard limit of discharge effluent as per Bureau of Indian standards.

Table 1: The Characteristics Of Textile Waste Water And Their Standard Values

S. No.	Parameters	Values (Eswaramoorthi et al., 2008)	Standards Values (BSI)
1	pH	6.0-10.0	5.5-9.0
2	Chemical oxygen demand (COD)(mg/L)	150-12,000	250
3	Biological oxygen demand (BOD)(mg/L)	80-6,000	30
4	Colour (Pt-Co)	50-2,500	None
5	Temperature (^{0} C)	35-45	50
6	Total Dissolved Solids (mg/L)	2,900-3,100	2100

Besides, increasing the pollutants load of ecosystem the cotton industry also uses a large amount of water as it is thought that about 200 L of water are used to produce 1 kg of textile.

Textile production process can be broadly divided into two categories: the spinning process (the dry process) and the wet process. Dry process includes separation of cotton fibers from the cotton seeds followed by spunning into cotton yarns (Sette et al., 1996). The cloths made up from cotton yarns undergo various wet processes like desizing, scouring, bleaching, dyeing etc. Dyeing is one of the most important steps in the wet process which involves changing the color of the fabric using dyes. Pectin imposed hydrophobicity in fabric opposes dyeing process. Thus, scouring is performed to remove pectin and other impurities through the use of alkaline baths (Ntuli et al., 2009). It consists of treating the cotton goods with 1- 2% of NaOH solution at high pressure and temperature (90-100°C) for 4-5 hours. Thus above treatment is not only leading to environmental pollution but also requires energy and water inputs in to the industry. It

contributes about 54% to the total BOD and is responsible for 10-25% of the total pollution load generated during the entire textile processing operation (Hannan et al., 2011). The chemicals of textile industry effluent cause various health related issues in human being and animals (e.g. respiratory, skin, digestive and cancer etc.). It also reduces crop productivity of soil (Rocky, 2012).

Bioscouring using pectinase enzyme may be a substitute for above chemical scouring which reduce effluent pollutants load and improves the quality of fabric. Pectinase is a general term which is used for a group of enzymes, such as pectin lyase, pectin methylesterase and polygalacturonase (Arunachalam and Asha, 2010). Pectin is a hetero–polysaccharide composed of 1, 4 linked galacturonate chains with a high percentage of methyl esterification (Yadav et al., 2007). Complete degradation of pectin requires the action of cocktail of enzymes which can be broadly classified into two groups (i) Methylesterases; which removes the methoxyl groups from pectin backbone and (ii) Depolymerases; which are responsible for the cleavage of bonds between different galacturonate subunits. This major group mainly includes the lyases and hydrolases. Lyases cleave the glycosidic bonds between the various subunits through trans-elimination and give rise to unsaturated products (Soriano et al., 2000). These are further classified into pectin lyase and pectate lyase according to their substrate specificity.

In cotton fiber, nearly 60% of the galacturonic acid residues of pectin are methyl esterified (Agrawal 2005). This methyl esterified pectin seems good target of pectin lyase enzyme. Literature reveals that in most of the bioscouring process pectate lyase and polygalacturonase were extensively used while pectin lyase is hardly explored and characterized. Further, the bioscouring process requires an enzyme which is active and stable over a wide range of pH and temperature. In addition to above, the process can be made economical at industrial scale by immobilizing the enzyme on suitable supports.

In the view of above concerns associated with cotton based textile industry, the present study will be carried out with the following objectives:-

- ✓ To isolate and identify pectin lyase producing microorganism form environmental samples.

- ✓ To characterize pectin lyase with respect to novelty.
- ✓ To purify pectin lyase enzyme and immobilize it on suitable supports.
- ✓ To compare the efficiency of pectin lyase mediated scouring with chemical scouring process.

Review literature

Chemical scouring of cotton is done with caustic alkaline solution at high temperature to achieve uniform dyeing and finishing. Although, the process is very effective in removing impurities, yet it utilizes huge amount of water for rinsing after the completion of process to remove the effect of alkali. Besides above, the process has a high energy requirement and yields waste products. The use of alkali toxifies the environment and as well as deteriorates the fiber by damaging the cellulose layer (Dhiman et al., 2013). Scanning electron microscopy of cotton fabric showed that the alkaline scouring deteriorates the fabric texture whereas bioscouring with biosurfactant plus pectinase gently removes hydrophobic impurities from the cotton fabric (Raza et al., 2014). Thus excessive usage of water and environment contamination is unacceptable (Dhiman et al., 2013).

Recently, research has been directed towards the discovery of environmental friendly enzymes that replace these chemical processes of scouring in textile industry with their high specificity for their substrates (Traore and Buschle–Diller, 1999). In contrast to drastic alkaline conditions conventionally used, treatment with pectin degrading enzymes would not affect the cellulose backbone and thus avoid fiber damage (Rouette, 2001). Pectinase appeared to be the most efficient enzyme for optimal scouring result (Wang et al., 2007) being capable of depolymerizing the pectin, breaking it down to low molecular water–soluble oligomers (Ahlawat et al., 2008) and thereby improving the absorbency and whiteness of the textile material.

In a study, thermostable pectinase of *B. subtilis* was used for bioscouring on desized cotton fabric and it resulted in enhancement in whiteness by 1.2 %, tensile strength by 1.6 % and tearness by 3.0 % as compared to traditional alkaline scouring (Ahlawat et al., 2009). Similarly, Rajandran et al., 2011 isolated a pectinase from *Fusariumn sp.* and its production was optimized under various cultural conditions. They partially purified pectinase enzyme and used it in the scouring of cotton fabric. They found that the water absorbing character and tensile strength of

the bioscoured fabric was considerably higher than that of the conventionally scoured fabric. In another study, Vigneshwaran et al., (2011) optimized bioscouring process variables (enzyme concentration, temperature and reaction time) and suggested that bioscouring of cotton fiber with pectinase should be done at 55- 60 ^{0}C for 45- 60 min with 5-6 % enzyme concentration for higher water absorbency and wax removal to get good yield.

Yuan et al., 2012(a) reported the gene cloning and expression of a low-temperature-active alkaline pectate lyase (PL D) from *Xanthomonas campestris* ACCC 10048. Recombinant PL D (r-PL D) produced in *E. coli* was purified by using Ni^{2+}-NTA affinity chromatography and showed an apparent molecular weight of ~38 kDa. The pH and temperature optima of r-PL D were found to be 9.0 and 30 °C, respectively. Compared with its microbial counterparts, r-PL D had higher activity over a wide pH range (>45 % of the maximum activity at pH 3.0-12.0) and at lower temperatures (>35 % of activity even at 0 °C). r-PL D showed similar efficacy, like commercial available pectinase (Novozymes), in reducing the intrinsic viscosity of polygalacturonic acid (35.1 % vs. 36.5 %) and in bioscouring of jute (10.25 % vs. 10.82 %). In another study done by Yuan et al., 2012(b) a pectate lyase gene (pl-str) was also cloned from *Streptomyces sp.* S27 and expressed in *E. coli*. Ni^{2+}-NTA purified recombinant enzyme (r-PL-STR) showed apparent molecular mass of ~35 kDa. The pH optimum of r-PL-STR was found to be 10.0, and it exhibited >70% of the maximal activity at pH 12.0 and retained more than 55% enzyme activity at pH 7.0-12.0 after incubation at 37°C for 1 hour. When combined with cellulase and α-amylase, r-PL-STR had comparable performance in bioscouring of jute fabric (22.39 vs. 22.99%).

Chiliveri and Linga, (2014) purified and characterized a novel thermostable pectate lyase from a UV mutant of *Bacillus tequilensis* SV11. The enzyme was found optimally active at 60 °C and pH 9. *Km* and *Vmax* of the purified pectate lyase was found to be 1.220 mg/ml and 1773 U/ml respectively. The enzyme was studied for its applicability in bioscouring and found to be efficient in the removal of 97.91% pectin of cotton fabric when compared with alkali-treated fabric. The pectinase was also applied along with biosurfactants in scouring process to obtain an increase in efficiency (Raza et al., 2014).

Methodology

- Isolation of microorganisms from environmental samples will be done by on defined media using serial dilution method (Aneja 2001), enrichment method (Patil and Chaudhari 2010).
- Screening will be done by using 1% CTAB (Patil and Chaudhari 2010).
- Isolates will be subjected for quantitative assay for pectin lyase activity (Miller 1959; Aguillar & Huitron 1990; Kashyap et al. 2000 and Singh and Mandal 2012).
- Identification of isolate will be done by using 16S rDNA method described by Oliwa-Stasiak et al. (2009) and Janda and Abbott (2007).
- Nutrients (C, N, P, trace elements etc.) and culture conditions (temperature, pH, time, agitation etc.) will be optimized and enzyme production will be carried out under optimized conditions (Kalaichelvan et al. 2013).
- Enzyme will be purified, immobilized on supports (e.g. polystyrene, silica gel etc.) and characterized for size, co-enzyme and cofactor requirements etc.
- The nucleotide sequence coding for pectin lyase will be amplified with designed primers and characterized at NCBI with the help of Bioinformatics programmes.
- Metagenomic approach will also be used to look for novel pectin lyase.
- Desized fabrics will be scoured through soluble pectin lyase and immobilized pectin lyase.
- Efficiency of bioscouring will be measured by weight loss method (Vigneswaran et al. 2012) and by determining amount of reducing sugar in bioscoured bath effluent (Cui et al. 2009).
- COD and BOD values of scoured bath for chemical scouring and bioscoruring will be characterized to determine ecofriendly nature of enzyme based process (Aneja, 2001).

Significance

The present study will help in management of cotton industry waste through bioscouring process. It will help in reducing the pollutants load of textile effluent, energy and water consumption. Immobilization of enzyme will help in improving the enzyme efficiency in various other industrial applications of pectin lyase such as fruit industry, paper and pulp, biofuel production, prebiotic/ functional food production etc.

References

1. Agrawal, P. B. (2005). PhD Thesis. The performance of cutinase and pectinase in cotton scouring. University of Twente.
2. Aguillar, G. and Huitron, C. (1990) Constitutive exopectinase produced by Aspergillus sp. CH-Y-1043 on di€erent carbon sources. *Biotechnology Letters* 12, 655-660.
3. Ahlawat, S., Dhiman, S.S., Battan, B., Mandhan, R.P. and Sharma, J. (2009) Pectinase production by *Bacillus subtilis* and its potential application in biopreparation of cotton and micropoly fabric. *Proc Biochem* 44, 521–526.
4. Ahlawat, S., Mandhan, R.P., Dhiman, S.S., Kumar, R. and Sharma, J. (2008) Potential application of alkaline pectinase from Bacillus subtilis SS in pulp and paper industry. *Appl Biochem Biotechnol* 149, 287-293.
5. Chiliveri, S. R., & Linga, V. R. (2014). A novel thermostable, alkaline pectate lyase from< i> Bacillus tequilensis</i> SV11 with potential in textile industry.*Carbohydrate Polymers*.
6. Cui, L., Wang, P., Wang, Q. and Fan, X. (2009). The bioscouring efficiency and activity of alkaline pectinase for cotton fabric. *Fibers and Polymers*, *10*(4), 476-480.
7. Dhiman, S.S., Mahajan, R. and Sharma, J. (2013) Pectinase of thermophilic microbes. In Thermophilic Microbes in Environmental and Industrial Biotechnology ed. Satyanarayana, T., Littlechild, J., Kawarabayasi, Y. pp. 689-710. Netherlands: Springer.
8. Eswaramoorthi S, Dhanapal K, Chauhan D (2008) Advanced in Textile Waste Water Treatment: The Case for UV-Ozonation and Membrane Bioreactor for Common Effluent Treatment Plants in Tirupur, Tamil Nadu, India. Environment with People's Involvement & Co-ordination in India. Coimbatore, India.
9. Ghaly AE, Ananthashankar R, Alhattab M, Ramakrishnan VV (2014) Production, Characterization and Treatment of Textile Effluents: A Critical Review. J Chem Eng Process Technol 5: 182. doi: 10.4172/2157-7048.1000182.
10. Hannan, M. A., Zahir, L., Zakaria, M., & Bhuiyan, A. H. (2011). Modification of Bioscouring Process by Addition of Cellulase Enzyme and Multifunctional Agents with Pectinase. *Cellulose*, *88*, 96.

11. Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of clinical microbiology*, *45*(9), 2761-2764.
12. Kalaichelvan, P. T. (2012). Production and Optimization of Pectinase from Bacillus sp. MFW7 using Cassava Waste. *Asian Journal of Plant Science and Research*, *2*(3), 369-375.
13. Kashyap, D. R., Chandra, S., Kaul, A., & Tewari, R. (2000). Production, purification and characterization of pectinase from a Bacillus sp. DT7. *World journal of Microbiology and Biotechnology*, *16*(3), 277-282.
14. Miller, L.G. 1959 Use of Dinitro-salicylic acid reagent for the determination of reducing sugars. Analytical Chemistry 31, 426-428.
15. Ntuli, F., Omoregbe, D. I., Kuipa, P. K., Muzenda, E., & Belaid, M. (2009, October). Characterization of effluent from textile wet finishing operations. World Congress on Engineering and Computer Science.
16. Oliwa-Stasiak, K., Molnar, C. I., Arshak, K., Bartoszcze, M., & Adley, C. C. (2010). Development of a PCR assay for identification of the Bacillus cereus group species. *Journal of applied microbiology*, *108*(1), 266-273.
17. Patil, Nitinkumar P., and Bhushan L. Chaudhari. "Production and Purification of Pectinase by Soil Isolate Penicillium Sp and Search for Better Agro-Residue for its SSF." *Recent Research in Science and Technology* 2.7 (2010).
18. Rajendran, R., Sundaram, S. K., Radhai, R., & Rajapriya, P. (2011). Bioscouring of cotton fabrics using pectinase enzyme its optimization and comparison with conventional scouring process. *Pakistan journal of biological sciences: PJBS*, *14*(9), 519-525.
19. Raza, Z. A., Rehman, A., Hussain, M. T., Masood, R., Saddique, M. T., Javid, A., & Ahmad, N. (2014). Production of rhamnolipid surfactant and its application in bioscouring of cotton fabric. *Carbohydrate research*, *391*, 97-105
20. Rocky, A. B. P. (2012). Comparison of effectiveness between conventional scouring & bio-scouring on cotton fabrics". International Journal of Scientific & Engineering Research. 3: 1-5.
21. Rouette HK. (2001). Encyclopedia of textile finishing. Springer, Berlin, pp 1–3, ISBN 3-540-65031-8

22. Sadasivam S. and Manickam A. (1996) Carbohydrate. In: Biochemical methods. New Age International, New Delhi.
23. Sette S, Boullart L, Langenhove LV (1996) Optimising A Production by A neural network/genetic Algorithm Approach. *Engng Applic Artif Intell* 9: 681-689.
24. Singh, S., & Mandal, S. K. (2012). Optimization of Processing Parameters for Production of Pectinolytic Enzymes from Fermented Pineapple Residue of Mixed Aspergillus species. *Jordan Journal of Biological Sciences*, *5*(4). 307-314.
25. Soriano, M., Blanco, A., Díaz, P. and Pastor, F. J. (2000). An unusual pectate lyase from a *Bacillus sp.* with high activity on pectin: cloning and characterization. *Microbiology*, *146*(1), 89-95.
26. Traore, M., and Buschle–Diller, G. 1999. Environmentally Friendly Scouring Processes. In: Book of papers of the Int. Conference and Exhibition of the AATCC, Charlotte, USA, pp. 183–189.
27. Vigneswaran, C., Ananthasubramanian, M., & Anbumani, N. (2012). Neural Network Approach for Optimizing the Bioscouring Performance of Organic Cotton Fabric through Aerodynamic System. *Journal of Textile and Apparel, Technology and Management*, *7*(3).
28. Wang, Q., Fan, X–R, Huab, Z–Z and Chen, J. (2007). Optimizing bioscouring condition of cotton kintted fabrics with alkaline pectinase from *Bacillus subtilis*. *Biochem. Eng. J.* 34: 107–113.
29. Yadav, S., Yadav, P. K. and Yadav, K. D. S. (2007). Pectin lyases of a few indigenous fungal strains. *Journal of scientific and industrial research*, 66(8), 601.
30. Yuan, P., Meng, K., Wang, Y., Luo, H., Shi, P., Huang, H., Tu, T., Yang, P. and Yao, B. (2012a). A low-temperature-active alkaline pectate lyase from Xanthomonas campestris ACCC 10048 with high activity over a wide pH range. *Applied biochemistry and biotechnology*, *168*(6), 1489-1500.
31. Yuan, P., Meng, K., Shi, P., Luo, H., Huang, H., Tu, T., Yang, P. and Yao, B. (2012b). An alkaline-active and alkali-stable pectate lyase from Streptomyces sp. S27 with potential in textile industry. *Journal of industrial microbiology & biotechnology*, *39*(6), 909-915.